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Role of macrophages in uveal melanoma

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Abstract

Tumor-associated macrophages have been related to a worse prognosis for survival in several tumors, among them uveal melanoma. In particular for proangiogenic and anti-inflammatory M2-type macrophages, a contributory role to tumor growth has been described. This study demonstrated that most tumor-associated macrophages in uveal melanoma exhibited the M2-phenotype. Tumors with monosomy 3 that have an unfavorable prognosis exhibited significantly more M2-type macrophages than tumors with disomy of chromosome 3. These findings point to a possible pathophysiologic mechanism that links an inflammatory phenotype in uveal melanoma with structural chromosomal abnormalities such as monosomy 3.

Keywords

CD163; CD68; inflammation; macrophages; monosomy 3; survival; uveal melanoma

In the following article, a paper investigating the role of macrophages in uveal melanoma has been critically evaluated. Uveal melanoma is the most frequently occurring primary intraocular malignancy. The tumor can spread hematogenously to the liver (and rarely to the lung) causing a fatal outcome with only limited therapeutic options. However, undetectable micrometastases can remain dormant for a long period of time and often form before the primary tumor is detected [1]. The ocular immune system in general and its role in tumor biology has been intensely studied, leading to the discovery of the ocular immune privilege incorporating the phenomenon of anterior chamber-associated immune deviation [2,3]. Mechanisms behind ocular immune privilege acting to minimize the expression of immunopathology aims to preserve vision while protecting the eye against pathogens [4]. For several diseases, such as uveal melanoma, cells contributing to the immune response thus became of interest. After an inflammatory phenotype had been associated with a poor outcome in uveal melanoma [5], macrophages were also detected as a negative prognostic factor for survival [6]. Further research pointed out that these so-called tumor associated macrophages (TAMs) are also significantly associated with monosomy of chromosome 3 [7].

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Macrophages derived from monocytes are a heterogeneous population of cells: resident (tissue) macrophages are present in several tissues in the human body, for example, in the liver where they are called Kupffer cells. Nonresident macrophages develop from circulating monocytes that are attracted to sites of, for example, acute inflammation.

Macrophages perform different tasks, such as scavenger function, tissue remodeling and wound healing, as well as participation in innate and adaptive immunity as antigen-presenting cells and regulators of immune responses. Depending on their differentiation, activation state or response to external influences in their microenvironment, the expression of surface proteins and other molecules (e.g., receptors) varies among different macrophage populations, reflecting their heterogeneity [8,9]. Recently, different polarized macrophages have been described, namely M1-type and M2-type macrophages [10]. Classically activated M1 macrophages that express CD68 (but not CD163) have immunostimulatory functions and can be antibacterial, anti-tumorigenic and anti-angiogenic. By contrast, alternatively activated M2 macrophages contribute to wound-healing processes, angiogenesis and debris removal (scavenger function), and are thus anti-inflammatory (immune suppressive) and proangiogenic. Fully polarized M1 and M2 macrophages represent extremes of a continuum of functional states [10].

The article under evaluation was able to demonstrate that TAMs in uveal melanoma consist predominantly of the M2 phenotype and are associated with an unfavorable prognosis, as shown for tumors elsewhere in the body.

Summary of methods & results

The authors investigated 43 tissue specimens of eyes enucleated for uveal melanoma between 1999 and 2004 [11]. Patients' charts and a central cancer databank were screened for information regarding metastatic disease and survival. A follow-up of 59 months (range: 14–121 months) was achieved. In addition, all tumors were staged based on histopathological criteria in accordance with the seventh edition American Joint Committee on Cancer (AJCC)–Union for International Cancer Control (UICC) eye cancer-staging system. Immunofluorescence double staining for the macrophage markers CD68 and CD163 was performed. The specimens were assessed for histologic parameters such as cell type and infiltrative behavior. CD34 stains for analysis of the microvascular density and FISH analysis for chromosome 3 status were also performed.

The immunofluorescence double staining was photodocumented with a confocal laser scanning microscope. The macrophage labeling was analyzed by counting the number of pixels for the single and double stains with a software analysis program. A total of 15 slides were evaluated manually and the results corresponded well to digitalized counting. Appropriate statistical analyses were performed to confirm the significance ($p < 0.05$) of the results.

Analysis of 43 uveal melanoma specimens showed a diffuse distribution of macrophages within the tumor. With the double immunofluorescence staining for CD68 and CD163, a higher number of macrophages revealed a coexpression of these markers and were thus attributed to M2-type macrophages. However, with regard to the distribution of CD68 and CD163 staining a large variability between the tumors was observed. Monosomy of chromosome 3 turned out to be significantly associated with a higher CD68, CD163 and CD68–CD163 double staining, compared with tumors with disomy of chromosome 3. A positive association between macrophages and other parameters for a poor outcome, such as ciliary body involvement, largest basal diameter and mean vascular density, were also found.

Kaplan–Meier survival analysis revealed that a low CD68 or CD68–CD163 staining pattern was associated with a significantly better survival. However, in contrast to largest basal diameter, ciliary body involvement, high MVD and monosomy 3, macrophage staining could not be evaluated as a significant predictor of death due to metastatic disease.

Expert commentary

In the majority of tumors investigated to date, M2 macrophages were associated with a reduced patient survival, for example, in ovarian cancer [12], pancreatic cancer [13] and skin melanoma [14]. The thorough conduction of this study allowed the authors to show that the same pattern applies to uveal melanoma. However, a figure with immunofluorescence double staining to illustrate the overall distribution of TAMs at different magnifications is missing. Based on good clinical data, an association of M2-type macrophages with monosomy 3 and thus an unfavorable prognosis for survival was found, suggesting that a pathophysiologic mechanism linking macrophage polarization to monosomy of chromosome 3 might exist.

Several interactions between macrophages and tumors have been already discovered [9]: TAMs exhibit several protumorigenic functions through the expression of manifold factors contributing to angiogenesis (via VEGF secretion), cell proliferation via production of growth factors, matrix remodeling and immune suppression [15]. In a mouse model of breast cancer, macrophages were able to induce an angiogenic switch accompanied by progression to malignancy [16]. Vice versa, *in vitro* studies in ovarian cancer cells were able to show that tumor cells can orchestrate macrophage polarization [17]. The NF- κ B signaling pathway that plays a role in several conditions, such as inflammation and carcinogenesis, was also found to be involved in macrophage polarization [18,19]. The tumor microenvironment is also contributory to many physiological processes, such as monocyte recruitment in general, or macrophage attraction at hypoxic sites [19,20]. However, the detailed pathophysiological interactions between tumors with respect to their microenvironment and macrophages, as well as therapeutic modifications, are still under investigation.

Five-year view

Over the next 5 years, further pathophysiologic interactions (and their related pathways) between macrophages and tumor cells will be detected. Researchers will also focus on the role of macrophages on liver metastasis. As liver metastases from uveal melanoma can remain dormant for a long period of time before they start to grow and become clinically detectable, macrophages and other factors supposedly contributing to the hepatic microenvironment of micrometastases will be investigated for their role in this scenario. Adjuvant therapeutic strategies, targeting in particular liver metastases and allowing for modification of the tumor microenvironment and its immunologic properties, may evolve from this research.

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Key issues

- Uveal melanoma is the most frequently occurring primary tumor in the eye.
- The prognosis for survival in uveal melanoma is dependent on the development of hepatic metastasis.
- Several prognostic factors are associated with an unfavorable prognosis for survival, such as monosomy 3, age, a high microvascular density, and an inflammatory phenotype (including macrophages).
- Tumor-associated macrophages (TAMs) contribute to tumor growth and angiogenesis.
- The study under evaluation demonstrated that TAMs predominantly consist of M2-type macrophages that display anti-inflammatory (immune suppressive) and proangiogenic properties.
- Tumors with the prognostic unfavorable monosomy of chromosome 3 consisted of significantly more M2-type macrophages than their disomy 3 counterparts.
- The underlying pathophysiological mechanisms require further investigation.